BRIEFER ARTICLES

OXYGEN PRESSURE AND THE GERMINATION OF XANTHIUM SEEDS

A PRELIMINARY REPORT

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 130

Progress in the analysis of the conditions which cause delayed germination in seeds has been disappointingly slow, largely due to the fact that investigators have persisted in neglecting the seed coat as a factor on account of its thinness, as in KINZEL'S recent paper,[1] or because of a preconceived idea that the embryonic protoplasm is more or less dormant in newly ripened seeds, as in FISCHER'S work,[2] and that some stimulus is necessary to activate it.

It has been shown by CROCKER[3] that the seed coat of Xanthium, in spite of its thinness, is the sole cause of delay under normal germinative conditions. He has also shown[4] that the delayed germination in seeds of aquatic plants depends in many instances on the seed coats. It is evident that the testa cannot be neglected as a factor until its insignificance in that rôle has been proven. If seeds are tested with coats removed, it is possible that no dormant protoplasm will be found needing ionic or other stimulus to growth. Investigations in which the seed coat has been arbitrarily neglected for any reason will have to be repeated before a satisfactory interpretation can be attempted.

On the other hand, it is certain that there are cases in which the delay must be attributed to protoplasmic characters, as in Crataegus, the testa of whose seeds has been proven not to be the cause of the delay in germination. The conditions for protoplasmic activity in the embryo of plants have not been analyzed carefully. A series of experiments is being conducted with the seeds of *Xanthium pennsylvanicum* to determine the exact

[1] KINZEL, WILHELM, Lichtkeimung. Einige bestätigende und ergänzende Bemerkungen zu den vorläufigen Mittheilungen von 1907 und 1908. Ber. Deutsch. Bot. Gesells. **26a**:631–645. 1908.

[2] FISCHER, ALFRED, Wasserstoff und Hydroxylionen als Keimungsreize. Ber. Deutsch. Bot. Gesells. **25**:108–122. 1907.

[3] CROCKER, WILLIAM, Rôle of seed coats in delayed germination. BOT. GAZETTE **42**:265–291. 1906.

[4] ———, Germination of seeds of water plants. BOT. GAZETTE **44**:375–380. 1907.

amount of oxgyen necessary for germination with the coats removed. TAKAHASHI[5] has shown that rice germinates in total absence of free oxygen, and CROCKER[6] has shown the same to be true for *Alisma Plantago* and Eichhornia. But the seeds of Xanthium with coats removed remain dormant if oxygen is entirely excluded, though all other germinative conditions are supplied.

The oxygen pressure necessary for the germination of seeds of *X. pennsylvanicum* has been determined with apparatus similar to that used by SCHAIBLE,[7] with modifications to exclude light and to control the temperature. The seeds are soaked in ice water for twelve hours, and the coats carefully removed, thus excluding them as a factor. The temperature was uncontrolled during the first experiments, but it was found immediately that high temperatures would yield results differing from those at low temperatures. The jars, therefore, were kept in a water bath with cold water running through it constantly. The variation in temperature was not more than about two degrees during the time of each experiment. The seeds used at pressures of less than 99^{mm} of mercury were collected in the spring, after lying in the field nearly six months. Those at 99^{mm} and above were collected in the fall as soon as ripe, and were kept in an unheated dry room during the winter and succeeding spring. Each lot of seeds was put on wet absorbent cotton and was subjected to certain conditions of pressure for ten days. The elongation of the hypocotyl, followed by the geotropic response, was used as a criterion of germination.

Since the desired oxygen pressure is secured by a reduction of total atmospheric pressure, the question naturally arises whether the reduction of pressure itself has any influence on the germination. Experiments are being conducted using the same oxygen pressures at full atmospheric pressure to determine whether the mere difference in pressure is a factor. As some time must elapse before these can be continued, I present the results of the first series of experiments in the accompanying table.

The effect of high temperature is seen by comparing the two experiments at 72^{mm}. The experiment at 99^{mm} was conducted with seeds that had been kept in the laboratory over winter, and the temperature averaged nearly 2° lower than the one at 90^{mm}, so that the percentage of germination was slightly less than at 90^{mm}, in spite of the increased oxygen pressure.

[5] TAKAHASHI, T., Is germination possible in absence of air? Bull. Coll. Agr. Tokyo **6**:439–442. 1905.

[6] CROCKER, WILLIAM, Longevity of seeds. BOT. GAZETTE **47**:69–72. 1909.

[7] SCHAIBLE, FR., Physiologische Experimente über das Wachstum und die Keimung einiger Pflanzen unter vermindertem Luftdruck. Beiträge Wiss. Bot. **4**:93–148. 1900.

It is perfectly clear from the figures given that the oxygen pressure necessary for germination is quite low, and that the pressure is not the same for the two seeds. The uppers require a higher pressure than the lowers; this is a real physiologic difference between the two seeds. It must be noticed that the difference in the embryo in the two seeds is in the same direction as the difference in their seed coats, both sets of characters acting in conjunction, not in opposition, in causing a longer delay in the uppers than in the lowers. However, the difference is so slight in the embryonic characters that the germination of the uppers is not at all hindered if the seed coats are off, with full atmospheric pressure. The uppers begin to germinate on the average just a few hours later than the lowers under such conditions.

Atmospheric Pressure	Oxygen Pressure	Temperature	Percentage germ. in 10 days				Growth in length of hypocotyl in 10 days (mm)			
			Lowers	Control	Uppers	Control	Lowers	Control	Uppers	Control
99mm	20.72mm	19–22°	75	100	45	95	14.5	30.0	4.9	23.3
90	18.84	21–22.6	80	95	50	100	22.8	45.9	4.3	37.8
*72	15.07	20–28	45	100	20	100	11.5	46.0	9.4	33.6
72	15.07	20–22	30	95	0	100	6.36	28.5	0.0	22.0
*28	5.86	21.5–24.5	0	100	0	95	00.0	37.8	0.0	28.8

*Temperature not controlled.

The seeds which failed to germinate under the experimental conditions of pressure and moisture were in every case brought into normal germinating conditions at the close of each experiment. Germination of 100 per cent. in nearly every instance shows that the seeds are not injured by the experimental conditions.

The surprising feature of the results is the small amount of oxygen pressure necessary for germination. From the rapid exchange of gases which Crocker has shown takes place in the seeds of the cocklebur, one would expect to find a rather high pressure required. The results I have obtained are inconsistent with the rapid respiration which he has shown to occur.

Two things must be taken into consideration in regard to this apparent contradiction of results. In the first place, the seed coats are probably responsible for a large amount of the respiration observed in the seeds of Xanthium with the coats intact. Becquerel[8] has shown that the integuments of seeds produce CO^2 quite freely, often showing a larger output than the seeds from which they are taken.

[8] Becquerel, Paul, Recherches sur la vie latente de graines. Ann. Sci. Nat. Bot. IX. 5: 193–320. 1907.

Moreover, there is a strong correlation between the growth of the hypocotyl and cotyledons. Normally the former grows first, and the latter do not enlarge until the root is well established. But if the seeds with coats on are placed in an atmosphere composed largely of oxygen, this normal correlation is reversed, the cotyledons elongating before the hypocotyl begins to develop. The testa is comparatively thick over the hypocotyl, very thin over the cotyledons, and certainly admits oxygen more quickly to the cotyledons than to the hypocotyl. The cotyledons are less sensitive than the other parts of the embryo, and require more oxygen to activate them than would be necessary for the hypocotyl. It is perfectly clear, then, that much of the oxygen used by seeds which germinate with the seed coats intact and in high oxgyen pressure is due to consumption of oxygen by the seed coats and the cotyledons, very little being used by the hypocotyls. In my experiments the pressure has been determined for the very sensitive hypocotyl, which always grows first if the coat is off, and the pressure required is low. I believe that these two points fully explain the difference in oxygen pressure necessary to germination with coats off and coats intact.

Further work is necessary to determine the exact relation of temperature to the oxygen pressures required, and series at high temperatures will be compared with series at low temperatures to obtain definite data on this point.

Fresh seeds will be collected this fall and tested immediately after they have ripened, to determine whether there is any after-ripening, whether the oxygen pressure necessary for germination is greater or smaller before the period of drying, freezing, and resting than it is later.

Acknowledgments are due to Dr. WILLIAM CROCKER, under whose direction the work here recorded was done.—CHAS. A. SHULL, *Transylvania University, Lexington, Ky.*